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Weight of the Evidence on the Human Carcinogenicity of 2,4-D*

M. A. Ibrahim, G. G. Bond, T. A. Burke, P. Cole, F. N. Dost, P. E. Enterline, M. Gough, R. S. Greenberg, W. E. Halperin, E. McConnell, I. C. Munro, J. A. Swenberg, S. H. Zahm, and J. D. Graham

The phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is widely used to control the growth of weeds and broadleaf plants. We convened a panel of 13 scientists to weigh the evidence on the human carcinogenicity of 2,4-D. The panel based its findings on a review of the toxicological and epidemiological literature on 2,4-D and related phenoxy herbicides. The toxicological data do not provide a strong basis for predicting that 2,4-D is a human carcinogen. Although a cause–effect relationship is far from being established, the epidemiological evidence for an association between exposure to 2,4-D and non–Hodgkin's lymphoma is suggestive and requires further investigation. There is little evidence of an association between use of 2,4-D and soft-tissue sarcoma or Hodgkin's disease, and no evidence of an association between 2,4-D use and any other form of cancer. Scientists on the panel were asked to categorize 2,4-D as a "known," "probable," "possible," or "unlikely" carcinogen or as a noncarcinogen in humans. The predominant opinion among the panel members was that the weight of the evidence indicates that it is possible that exposure to 2,4-D can cause cancer in humans, although not all of the panelists believed the possibility was equally likely: one thought the possibility was strong, leaning toward probable, and five thought the possibility was remote, leaning toward unlikely. Two panelists believed it unlikely that 2,4-D can cause cancer in humans.

Preface

2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most widely used herbicides today. In light of the growing body of scientific data on the substance, which includes new animal and human studies, the health of those who are exposed to 2,4-D has been of increasing concern to the Environmental Protection Agency (EPA), Congress, and state agencies.

In 1989, John D. Graham of the Harvard School of Public Health convened a panel of scientists to examine the weight of the scientific evidence on the potential carcinogenicity of 2,4-D. Financial support for the workshop was provided by the Industry Task Force II on 2,4-D Research Data (an association of manufacturers and commercial formulators of 2,4-D) through a grant to the National Association of Wheat Growers Foundation.

The members of the panel had expertise in epidemiology, toxicology, exposure assessment, and industrial hygiene. The ground rules for the panel members called for reviewing published data, considering all relevant evidence, and making weight-of-the-evidence judgments. The panel was not expected to reach a consensus, and efforts were made in this report to capture differences in expert opinion.

*See appendix for author affiliations.
Address reprint requests to J. D. Graham, Center for Risk Analysis, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115.

2,4-D Uses and Exposure

The chlorophenoxy herbicide 2,4-D is prepared by the condensation of 2,4-dichlorophenol and monochloroacetic acid. The acid is the parent compound, but almost all of the 2,4-D formulations in use contain the amine salts or the alkali salts, which are more water soluble than the acid, or the ester derivative, which is readily dissolved in an organic solvent. Other herbicide structural analogs of 2,4-D ("phenoxy herbicides") include the butyric acid (2,4-DB) and the propionic acid (2,4-DP) derivatives; 4-chloro-2-methyl-phenoxyacetic acid (MCPA); 4-chloro-2-methylphenoxy propionic acid (MCPP); and the once commonly used herbicides 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichlorophenoxy) propionic acid (Silvex, 2,4,5-TP).

Overall, the 2,4-D technical grade formulations are approximately 90 to 99% pure (*1*). The most common impurities of technical grade 2,4-D include other phenoxyacetic acids, a variety of chlorinated phenols, and possibly low levels of nitrosamines in the amine salts. Trace amounts of polychlorinated dibenzo-*p*-dioxins (PCDD) have been identified in the amine and ester formulations, with the 2,7-CDD, tri-, and tetra-dioxin isomers the most commonly detected (*2*). The highly toxic dioxin 2,3,7,8-TCDD has not been identified in 2,4-D formulations, nor have other dioxins with chlorine atoms at the 2,3,7, or 8 positions (*1,3*). The current toxic equivalency factor (TEF) methodology

proposed by the U.S. Environmental Protection Agency (EPA) indicates that the tetra-CDDs other than the 2,3,7,8 congener have a relative toxicity of 0.01 compared to 2,3,7,8-TCDD, whereas the di- and trichlorinated PCDD isomers are considered to exhibit zero toxicity (*4*).

Commercial use of 2,4-D in the United States increased rapidly following World War II (*3*). The 2,4-D amine and alkali salts and ester formulations are used to control the growth of broadleaf plants and weeds on range lands, lawns, golf courses, forests, roadways, parks, and agricultural land. 2,4-D is preferred over other herbicides for several reasons: low cost, effective action, and low acute toxicity. The various 2,4-D compounds are relatively mobile in most soils and are absorbed through both the roots and leaves of most plants, especially broadleaf species (*5*).

The studies documenting potential human exposures to 2,4-D have focused on occupational cohorts and have identified the highest exposure levels in occupations involving the formulation or use of 2,4-D. Residential use of 2,4-D formulations typically produce much lower exposures than those observed in the occupational cohorts.

The studies completed for cohorts spraying 2,4-D and other phenoxy herbicides suggest that the level of exposure is a direct function of the rate of application (*3*). Individuals using backpack sprayers on right-of-ways receive the potentially highest daily personal exposures (3.4–4.9 mg/day) followed by helicopter and airplane application personnel (0.005–1.04 mg/day), farmers driving tractors (0.48 mg/day), and hand and tank commercial lawn sprayers (0.29 mg/day) (*6–9*). Variations in the estimated exposure levels are presumed to be based on the variable workplace setting and the amount of protective gear worn by the various occupational cohorts (*3*). Estimates of the total lifetime doses are related directly to the number of days per year and the number of years of spraying activities. Experimental data from Grover et al. (*7*) and Lavy (*10*) indicate that exposure levels may be reduced by better workplace practices, such as wearing gloves and changing clothes following spraying activities. Although noncommercial applicators' exposure levels have not been studied, the homeowner who occasionally uses 2,4-D for domestic applications most likely receives daily doses and total lifetime exposures far lower than the occupational cohorts.

Toxicology

In this section, the toxicological evidence on 2,4-D is reviewed, including information on how the chemical is metabolized, its acute toxicity, the results from mutagenicity tests, and the findings from long-term carcinogen bioassays in animals. In performing this review, workshop participants relied primarily on recent reviews produced by the World Health Organization, the Canadian Centre for Toxicology, the International Agency for Research on Cancer, and the U.S. EPA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (*1,3,11,12*). Virtually all of the toxicology studies have been done on the 2,4-D acid, and the assumption has been that these data apply to the 2,4-D esters and amines because these materials are readily metabolized to the acid.

Metabolism

Several reviews have been published regarding the absorption, distribution, metabolism, and excretion of 2,4-D in humans and other mammals (*1,3,13*). The salient metabolic information relevant to evaluating the current weight of evidence of human carcinogenicity for 2,4-D is summarized below.

Data collected from occupational exposure studies indicate that 2,4-D may be absorbed at varying rates via the three classic exposure routes: inhalation, ingestion, and dermal absorption. Although the percent of 2,4-D applied to the skin that is absorbed has been shown to be low in humans (5.8–6.4%) compared to the dermal absorption of some other organic chemicals, the dermal absorption exposure route accounts for approximately 90% of the total absorbed dose of 2,4-D in workers who spray herbicides. The inhalation route of exposure is more important among workers who manufacture 2,4-D (*1,14,15*).

Based on the results of ingestion uptake studies in human volunteers, 2,4-D administered in single doses is absorbed rapidly and completely (*16*). Although uptake via inhalation of 2,4-D in humans has been poorly studied, respiratory uptake in the rat indicates that 2,4-D is rapidly absorbed. Data from studies of applicators who spray 2,4-D indicate that respiratory exposures typically contribute only 2% of the total 2,4-D body burden (*1,7*).

Studies of human subjects following oral exposure indicate that 2,4-D is rapidly absorbed and carried in the bloodstream throughout the entire body (*16*). The metabolic and excretory patterns of 2,4-D in humans have been studied in both volunteers and occupational cohorts. The results from volunteers receiving single doses show that 2,4-D is rapidly excreted in the urine and has a biological half-life of approximately 18 to 20 hr, as approximately 75 to 90% of an absorbed dose is excreted within 4 days (*1,15,16*). A half-life in humans based on multiple 2,4-D doses has not been estimated with certainty.

The rates of formation of 2,4-D metabolic products are highly variable in humans. The majority of absorbed 2,4-D is eliminated in the urine as the original material without undergoing any metabolic alterations (*1,16*). Sauerhoff et al. have identified 2,4-D conjugates in humans ingesting pure 2,4-D with a formation rate as high as 27% (*16*). No evidence on the human toxicity of these conjugates and other metabolites of 2,4-D has been identified in the literature.

Acute Toxicity

The published laboratory animal studies on the acute toxicity of 2,4-D indicate that it is a moderately toxic compound (*1*).

The LD_{50} values for various 2,4-D analogs are highly dependent on the chosen animal species (*1*). Dogs, for example, are known to excrete organic acids poorly compared to rodents and humans. Oral LD_{50} values for the 2,4-D acid range from 100 mg/kg for the dog to approximately 1000 mg/kg for the guinea pig. The ester formulations have oral LD_{50} values ranging from 380 mg/kg for the mouse to 2960 mg/kg for the chicken, and the sodium salt form has oral LD_{50} values ranging from 375 mg/kg for the mouse to 2000 mg/kg for the rat. These studies suggest that the dog is the mammalian species most sensitive to acute oral 2,4-D exposures (LD_{50}=100 mg/kg), while the majority of the oral LD_{50} values range from 300 mg/kg to 1000 mg/kg. Humans ingesting 2,4-D have survived doses as high as 100 mg/kg, suggesting that the LD_{50} for humans may be in the same range observed in the other animal species.

Results from studies of 2,4-D indicate that the dog has a subchronic no-observable-effect level (NOEL) of 10 mg/kg/day, and

the rat has a NOEL of 30 mg/kg/day (*1*). A NOEL for reproductive effects in pregnant rats was observed at 10 mg/kg/day (*1*). Offspring exposed to higher 2,4-D doses *in utero* experienced decreased birth weight without apparent maternal toxicity.

The primary symptoms of acute toxicity to 2,4-D exposure include damage to muscle tissue and the gastrointestinal tract and depression of the central nervous system (CNS) (*3*). A study published recently, however, did not find any peripheral neuropathy effects in rats treated dermally for 3 weeks with a 12% 2,4-D amine mixture and in rats treated for 2 weeks with a 24% 2,4-D acid mixture (*17,18*). This animal study does not support the limited case study evidence that acute exposures to 2,4-D are associated with peripheral neuropathy neuropathy in humans. The World Health Organization (WHO) has questioned the peripheral neuropathy finding because individuals given 2,4-D or 2,4-D mixtures did not exhibit this adverse effect. WHO suggests that the neuropathy effects possibly resulted from exposure to other organic compounds, from poor nutrition, or from heredity. More studies involving acute human exposure to 2,4-D will be required to assess this potential health effect (*1*).

Mutagenicity

Although it has been one of the most rigorously tested compounds, the available evidence on the mutagenicity of 2,4-D and its related products is equivocal to negative. The evidence indicates that 2,4-D does not exhibit the gene-damaging potential of a classic mutagen. For example, 2,4-D is not mutagenic in the Ames Salmonella test nor in *E. coli*, both commonly used strains in *in vitro* tests. Other *in vitro* tests conducted by a variety of investigators have demonstrated both positive and negative results, and hence the overall evidence remains equivocal (*3*).

In vitro, 2,4-D has caused sister chromatid exchanges (SCEs) in cultured human lymphocyte cells, but the majority of the SCE *in vivo* assays in rats, mice, hamsters, and humans has been negative (*19,20*). Positive mutagenic effects have been noted in *in vivo* tests of mouse bone marrow at doses of 100 mg/kg and 300 mg/kg, which are near LD_{50} does, but no mutations were observed at the 10 mg/kg and 50 mg/kg levels.

For the purpose of evaluating the mutagenicity of 2,4-D in humans, it should be noted that the lymphocyte target cells in the positive *in vitro* study are similar to the cancer sites in epidemiological studies. Although the negative results of *in vivo* studies of lymphocytes and bone marrow cells makes the significance of this association questionable, it still merits consideration.

Carcinogen Bioassays

The laboratory animal bioassays of 2,4-D published before 1986 have been considered inadequate because they do not meet current experimental bioassay guidelines published by the Inter national Agency for Research on Cancer (IARC), the WHO, and the EPA (*1,11,12,21*). An EPA recommendation that additional animal cancer bioassays be conducted prompted the Industry Task Force on 2,4-D Research Data to sponsor new studies in rats and mice that were completed in 1986 and 1987, respectively (*22,23*).

In the first study, 5 groups of 60 male and 60 female Fischer 344 rats were exposed to 2,4-D in a chronic feeding study at doses of 0 (controls), 1, 5, 15, and 45 mg 2,4-D/kg body weight/day orally for 2 years (*23*). These dose groups were selected based on the results of a 13-week subchronic study indicating that 60 mg/kg/day doses produced kidney damage, thereby satisfying the requirement of a maximum tolerated dose (MTD). The male rats in the highest exposed group experienced a statistically significant increase in a particular type of brain tumor (astrocytoma), as six rats with astrocytomas were observed upon autopsy over the 2-year period. Two astrocytomas were detected in the 15 mg/kg/day male cohort; one such tumor was identified in the male controls. no statistically significant increases in the incidence of brain tumors were observed in the three groups of exposed male rats receiving the lowest doses or in any of the exposed groups of female Fischer rats.

In the second study, 4 groups of 60 male and 60 female $B6C3F_1$ mice were exposed to oral doses of 0, 1, 15, or 45 mg/kg/day of 2,4-D in their diet for 106 weeks (*22*). No excess incidence of tumors was observed in any of the groups of male or female mice in this study.

Considered together, these two animal studies do not provide impressive evidence that exposure to 2,4-D causes cancer in animals. Based on the results from the rat study, the workshop participants concluded that there was weak evidence supporting an excess of brain cancer occurrence in the male Fischer 344 rats receiving the highest dose. The evidence of a relationship between 2,4-D exposure and brain cancer was considered weak for several reasons. First, the spontaneous incidence of brain tumors in rats in the control groups of other bioassays has been shown to be somewhat variable (*24*). hence, there is some doubt about whether the excess incidence of brain tumors in the highest dose group of male rats is attributable to 2,4-D. (It should be noted, however, that most of the variability in brain tumor incidence was in strains other than Fischer 344.) Second, the female rats at the highest dose group did not exhibit an excess incidence of brain tumors. This is surprising because sex differences in metabolism, excretion, or permeation of the blood-brain barrier are not known to exist. Although the panel could not explain this observation, they speculated that in the male rat the dose of 45 mg/kg/day may saturate the capacity of both the kidney and the choroid plexus of the brain to excrete 2,4-D in rats at the higher doses. Several panel members stressed the importance of such a study. The additional carcinogenicity bioassay requested by the U.S. EPA will either replicate or fail to replicate the finding of tumors at high doses. If the tumor finding is replicated, pharmacokinetic investigations could test the hypothesis that high doses saturate the capacity of the excretory pathways for 2,4-D, resulting in tissue accumulations that may not be relevant for lower exposures.

Workshop participants noted that the bioassay of mice may not have achieved the MTD. The workshop participants had a somewhat different view of the MTD issue from either the EPA or the EPA's FIFRA Science Advisory Panel. EPA concluded that the MTD was not achieved in either rats or mice (*12*). The Science Advisory Panel concluded that the highest dose groups in both species were close enough to the MTD. Workshop participants felt that the EPA's criticism was more valid in the case of the mice that in the case of the rats. Additional animal experiments should be conducted to resolve the MTD issue and provide a rigorous test of the brain tumor hypothesis.

Epidemiology

The epidemiological studies of phenoxy herbicides and human cancer have employed both the cohort and case–control designs. Scientific reviews of the epidemiological evidence on herbicide use and human cancer are available in Smith and Bates (*25*), Blair and Zahm (*26*), Blair et al. (*27,28*), and Bond et al. (*29*). In this report, we focus on those studies that are most relevant to assessing the weight of the evidence on the carcinogenicity of 2,4-D. We begin with the case–control studies because findings from several initial studies have largely driven the epidemiological research on phenoxy herbicides, including 2,4-D. The findings are summarized collectively at the end of the section.

Case–Control Studies

In a series of studies initiated in the late 1970s, Hardell and co-workers examined whether exposures to various chemicals in Sweden were associated with soft-tissue sarcoma (STS), Hodgkin's disease (HD), and non–Hodgkins's lymphoma (NHL). Significant associations between phenoxy herbicide use and the three types of cancer were reported in several published papers (*30–33*). Hardell and co-workers advanced various biological hypotheses to explain the associations they observed.

Some participants thought that the studies by Hardell and colleagues were not directly relevant to the workshop's assessment because they did not report any quantitative information about human exposure to 2,4-D. Moreover, Colton (*34*) has summarized numerous criticisms of the Hardell studies, including potential bias in the selection of cases and controls, potential recall bias due to media publicity about the hypothesis under study, and the inability to adjust for potentially confounding factors. The controversy surrounding the Hardell studies stimulated epidemiologists around the world to investigate the relationship between herbicide use and human cancer. Other participants believed that the Hardell studies warranted consideration in spite of these potential shortcomings. For example, one of the studies reported an STS odds ratio of 4.2 (confidence interval not specified) for the phenoxy herbicides excluding 2,4,5-T. It should be noted that other chemicals in addition to 2,4-D are encompassed by this classification, including MCPA, MCPP, and 2,4-DP (*31*). The panel decided to focus its attention on the more recent studies motivated in part by Hardell's work.

Smith et al. (*35*) studied STS in New Zealand where phenoxy herbicides (especially 2,4,5-T and 2,4-D) have been widely used since World War II. Eighty-two cases and 92 controls were selected from the National Cancer Registry for the years 1976 through 1980. A telephone survey was administered to either the STS patient or next of kin to determine whether subjects were potentially exposed to phenoxy herbicides and other chemicals. Specific information on the amount of exposure to 2,4-D was not reported.

Vineis et al. (*36*) studied STS in a region of Northern Italy where rice growing is widespread and phenoxy herbicides (2,4,5-T, 2,4-D, and MCPA) were used beginning in the 1950s. Telephone interviews or postal questionnaires were administered to 68 histologically confirmed cases of STS or their next of kin and to 158 controls drawn randomly from electoral rosters. Specific information about the amount of human exposure to 2,4-D was not reported in the study. Sales figures from the region indicate that although significant amounts of 2,4-D were used, 2,4,5-T was the most widely used phenoxy herbicide until 1970, when it was banned.

Pearce et al. (*37,38*) and Pearce (*39*) studied NHL and exposure to phenoxy herbicides in New Zealand. The expanded study included 183 male cases of NHL and 338 controls taken from the New Zealand Cancer Registry for the years 1977 through 1981. The telephone survey was similar in design to the one used by Smith et al. (*35*). No specific information on use of 2,4-D is reported in the study, although both 2,4,5-T and 2,4-D were widely used in New Zealand during the 1950 to 1989 period.

Hoar et al. (*40,41*) identified newly diagnosed cases of STS, HD, and NHL among males in Kansas for the period 1976 to 1982 through the University of Kansas Cancer Data Service. One hundred thirty-three STS cases, 121 HD cases, and 170 NHL cases were studied. Using a random telephone survey and Medicare files, three white males were selected as controls for each case, matching for age and vital status. Telephone interviews were conducted to obtain information on occupational factors and the frequency and duration of herbicide use. Information about the frequency and duration of 2,4-D use specifically was not collected. A separate survey of pesticide suppliers was used to corroborate evidence of self-reported pesticide exposure.

Woods et al. (*42*) studied STS and NHL in Washington State to explore possible associations between these cancers and the use of phenoxy herbicides and chlorinated phenols. A tumor registry was used to identify men diagnosed with either STS or NHL from 1981 through 1984. Controls were obtained by means of random digit dialing and random selection from Medicare files. Controls were matched to cases according to vital status and age. For analytical purposes, there were 128 STS cases, 576 NHL cases, and 694 controls. A detailed personal interview was administered to obtain information on job titles and job activities, which the authors used to assign subjects to four categories of exposure to phenoxy herbicides ("high," "medium," "low," or "no" exposure). The likelihood of exposure to pesticides based on the respondents' job titles or activities was the sole determinant in assigning them to an exposure category. Subjects were also asked about their use of specific herbicides, including 2,4,5-T and 2,4-D.

Zahm et al. (*43*) applied the same methods used in the earlier Kansas study (*40,41*) to study confirmed cases of NHL in eastern Nebraska. There were 385 cases (201 men, 184 women) and 1432 controls (725 men, 707 women) who completed the interviews. The distinctive feature of this study is the collection of specific information on the duration and frequency of 2,4-D use. Detailed information on the use of other herbicides was also collected.

A study of NHL cases and herbicide use has been conducted by the National Cancer Institute (NCI) in the states of Iowa and Minnesota. Data were being analyzed at the time of the workshop, and no results were made available to workshop participants.

The findings of each of the case-control studies are summarized below. Findings for each cancer type are discussed separately.

The findings for STS were not consistent among the studies. Vineis et al. (*36*) reported an elevated odds ratio (OR) of 2.7 (90% CI 0.59–12.37) for females exposed to phenoxy herbicides. Women under the age of 75 who were exposed in the 1950 to 1955 period had a higher OR of 15.5 (90% CI 1.3–180.3). Smith et al.

(*35*) reported a slightly elevated OR for males exposed to phenoxy herbicides, but the confidence limits were consistent with no association. Hoar et al. and Woods et al. found no association between use of phenoxy herbicides and STS. None of these studies reported an OR specifically for exposure to 2,4-D.

The only recent study of HD was performed by Hoar et al. (*40,41*). They found no association between use of phenoxy herbicides and HD in Kansas. Again, the study reported no OR specifically for 2,4-D exposure and HD.

The case–control findings for NHL, taken as a whole, suggest an association with use of phenoxy herbicides, although the evidence is not entirely consistent. Less clear but still suggestive is the evidence for an association between NHL and exposure to 2,4-D. The specific findings follow.

Hoar et al. (*40,41*) reported an NHL OR of 2.2 (95% CI 1.2–4.1) for subjects who had ever used phenoxyacetic acids (2,4-D and/or 2,4,5-T). Elevated ORs were also reported for several other herbicides. The association with phenoxy herbicides persisted after adjustment for use of other herbicides. When the analysis was restricted to phenoxy herbicide users who used only 2,4-D (i.e., eliminating users of 2,4,5-T), the OR increased to 2.6 (95% CI 1.4–5.0). The associations were particularly strong for those farmers who mixed and applied herbicides themselves and for those farmers who used backpack sprayers.

There was a significant increase in risk of NHL with increasing years of herbicide use and with number of days of herbicide exposure per year. Persons exposed to herbicides more than 20 days per year had an OR of 6.0 (95% CI 1.9–19.5). Persons who reported usually mixing or applying the herbicides themselves and who were exposed to the herbicides for more than 20 days per year had an OR of 8.0 (95% CI 2.3-27.9). Persons who were exposed to 2,4-D only and who were exposed more than 20 days per year had an OR of 7.6 (95% CI 1.8–32.3). The authors were careful to note that this OR could not be determined with complete accuracy because of the way in which the questionnaire elicited dates and frequency of herbicide use.

It should be noted that Hoar et al. (*40,41*) detected an independent 2-fold risk of NHL associated with fungicide use. This risk was statistically significant and persisted regardless of herbicide use. Furthermore, several other herbicide exposures (triazine, for instance) showed higher excess risk of NHL than 2,4-D but were not evaluated in as much detail as 2,4-D exposures. This raises the possibility that other exposures, not adequately controlled for, could have accounted for some of the observed association between 2,4-D and NHL.

Interestingly, Hoar et al. (*40,41*) report an association between NHL and failure to use protective equipment such as rubber gloves and masks (OR = 2.1; 95% CI 1.0–4.2). Because this study did not collect data on the frequency and duration of 2,4-D use specifically, it is not possible to estimate directly an association between the amount of exposure to 2,4-D and NHL.

The results from the Nebraska study by Zahm et al. (*43*) seem to support a specific association between 2,4-D use and NHL, although the magnitude of the OR was somewhat smaller than that reported in the Kansas study (even though the Kansas study did not report 2,4-D exposure specifically). Among men, mixing or applying 2,4-D was associated with an OR of 1.5 (95% CI 0.9–2.5). As the number of days per year mixing or applying 2,4-D increased, the OR tended to increase. Mixing or applying 2,4-D 21 days per year or more was associated with an OR of 3.3 (95% CI 0.5–22.1). There was, however, no association between the number of years 2,4-D was used on the farm and NHL ($p = 0.274$). There was a potential association between the number of days per year 2,4-D was mixed or applied and NHL ($p = 0.051$). Those farmers who usually changed their clothes immediately after handling 2,4-D had an OR of 1.1 (95% CI 0.4–3.1) compared to an OR of 1.5 (95% CI 0.8–2.6) for those who changed clothes at the end of the work day and an OR of 4.7 (95% CI 1.1–21.5) for those who waited until the following day or later to change clothes. This trend was statistically significant ($p = 0.015$). If these associations reflect a cause–effect relationship, they imply that changing clothes immediately after handling 2,4-D can reduce a farmer's risk of developing NHL.

Zahm et al. (*43*) also reported that adjustment for organophosphate use substantially lowered the estimates of risk for NHL associated with 2,4-D use. It was not possible to separate out the effects of 2,4-D and organophosphates among more frequent 2,4-D users because there were no cases exposed to 2,4-D for 21 or more days per year who were also unexposed to organophosphates.

The findings by Woods et al. (*42*) provide weak evidence of an association between phenoxy herbicides and NHL. In that study, four exposure categories were constructed based on job title or job activity. When a specific job title given in an interview suggested possible exposure to phenoxy herbicides, additional questions were asked to ascertain exposure to specific chemicals including 2,4-D (*42*). It was not clear to workshop participants that this interview method could provide a study population whose exposure could be controlled for all chemicals other than 2,4-D. Exposure to phenoxy herbicides was associated with an OR of 1.07 (95% CI 0.8–1.4) in all the specified occupations and an OR of 0.87 (95% CI 0.5–1.5) among the entire study population. The OR increased to 1.71 (95% CI 1.04–2.8) among those with cumulative exposures to phenoxy herbicides of more than 15 years concluding at least 15 years before diagnosis. The authors found no association between the intensity of phenoxy herbicide exposure and NHL using the four exposure categories. However, persons who worked regularly in jobs involving spraying weeds in forests had an OR of 4.8 (95% CI 1.2–19.4). All of these forestry sprayers reported using 2,4-D and 2,4,5-T combined, as well as preparations containing other chemicals. In addition, farmers who used phenoxy herbicides also had an increased risk for NHL (OR = 1.33; 95% CI 1.03–1.7). Persons who reported using 2,4-D specifically had an OR of 0.73 (95% CI 0.4–1.3), although, as stated above, it is difficult to determine if this OR was controlled for other chemical exposures. The negative association between 2,4-D use and NHL makes this possible failure to control for other exposures less troubling, as confounding exposures would tend to increase, rather than decrease, the OR.

Pearce et al. (*37,38*) reported little or no evidence of an association between use of phenoxy herbicides and NHL. The predominant phenoxy herbicide used in New Zealand is 2,4,5-T (*35,37*), and the use of 2,4-D is not well documented in these studies. There was no association between exposure to phenoxy herbicides and NHL, even for those with probable or definite exposure of at least 5 days more than 10 years before diagnosis (using the pooled cohorts, OR = 1.4; 90% CI 0.7–2.5). The specific occupations or activities involving potential exposure to phenoxy herbicides were also not associated with NHL. An updated report by Pearce (*39*)

found no association between NHL and frequency or duration of phenoxy herbicide use. The studies by Pearce and colleagues did not report any specific OR estimates for 2,4-D use.

Workshop participants discussed a variety of potential problems with the exposure information reported in the case–control studies, including inaccurate recall of herbicide use by all subjects and inaccurate recall of specific herbicides used. If such inaccuracies affect cases and controls equally (i.e., nondifferential misclassification), the net effect is to underestimate the strength of the herbicide–NHL association. It is likely that such problems occurred in all the studies to some extent and may explain the negative results in some studies. If cases had better recall of exposure than controls (e.g., due to media publicity about the association under study), then relative risks could be inflated due to differential misclassification. Most investigators took some steps to minimize this potential for bias (e.g., use of cancer cases as controls, blinding of interviewers to whether subjects were cases or controls, study of several types of cancer, and corroborative surveys of pesticide suppliers). Nonetheless, the possibility of differential misclassification cannot be ruled out in any of these studies.

Even if exposure information in case–control studies is accurate, there may be errors in the diagnosis of heterogenous diseases such as STS and NHL. Pathologic reviews can reduce, but not eliminate, misclassification of diseases. If misdiagnosis of cases is random, it will tend to suppress estimates of relative risk. This problem may be particularly acute for some of the studies of STS, but may also be relevant for NHL.

Several concerns were raised at the workshop about whether the elevated ORs reported by Zahm and co-workers (*43*) should be attributed to the specific use of 2,4-D. Although these concerns are not easily addressed in such studies, they need to be considered in any weight-of-evidence evaluation.

First, 2,4-D has been and continues to be used in combination with fertilizers and other herbicides, which can cause collinearity. Although attempts were made to adjust mathematically for other herbicide exposures, these attempts depend on the accuracy of the information provided by subjects on the use of specific herbicides. If the reporting of specific herbicide use by subjects (and next of kin) is subject to error, then the attempts to control statistically for use of specific herbicides may not be successful. One cannot dismiss the possibility that 2,4-D has been falsely implicated or that the ORs for 2,4-D are suppressed inappropriately when the adjustments are made for use of other herbicides.

Second, the methods of preparing and applying 2,4-D should be considered when interpreting the findings reported by Zahm and colleagues (*43*). For example, it was not uncommon in the past for users of 2,4-D to mix the herbicide with other chemicals such as kerosene and diesel oil; indeed, such practices continue today. Hence, it is possible that use of 2,4-D is serving as an indicator for other toxic agents that were commonly mixed with 2,4-D.

Third, it is possible that heavy use of 2,4-D is acting as an indicator for some unknown confounder that is responsible for the elevated risk of NHL. This interpretation is supported by the apparent association between exposures to pesticides other than 2,4-D and the increased risk of cancers in these studies. Possible confounding factors worthy of investigation include the socioeconomic status of applicators, the crops themselves, or other contaminating agents in the farm environment. [It should be noted that some of these potential confounders, e.g., crop type and animals, were found by Hoar et al. (*40,41*) not to be responsible for the observed association between increased cancer risk and exposure to 2,4-D.]

Finally, it is well known that 2,4-D has been produced over the years with varying amounts of impurities. It is not clear whether these impurities were present in large quantities or whether such impurities are sufficiently potent carcinogens to explain the results reported by Zahm and co-workers (*43*). Workshop participants thought it was unlikely that dioxin contamination alone could explain the cancer associations because 2,4-D does not contain 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, the most toxic dioxin isomer. The 2,4-D formulations do contain PCDD congeners, but these compounds are considered to be much less potentially carcinogenic than 2,3,7,8-TCDD. 2,4-D formulations do not contain significant quantities of other potentially carcinogenic compounds.

Cohort Studies

The workshop examined cohort studies by Lynge (*44*), Axelson et al. (*45*), Riihimaki et al. (*46*), Bond et al. (*47*), Green (*48*), and Wiklund et al. (*49*). Only the three studies that were judged to provide important findings for the weight-of-evidence evaluation are discussed here. The Axelson, Riihimaki, and Green studies are not included because of their small cohorts or their relatively low statistical power to demonstrate a potential effect. Based on the information presented in these papers, none of the studies excluded from the discussion included a single case of NHL or STS.

Bond et al. (*47*) examined 878 chemical workers exposed to 2,4-D during the 1945 to 1982 period, with follow-up extending through 1982. Observed mortality was compared with expected levels based on adjusted rates for white males from the United States and for other employees from the same manufacturing location who were not exposed to 2,4-D. Analyses by production area, duration of exposure, and cumulative dose showed no patterns suggestive of a cause–effect relationship between 2,4-D exposure and any particular cause of death. No cases of brain cancer or STS were observed in the cohort. One death from HD was reported. There were two deaths from NHL compared to 0.5 expected. These two workers had potential for exposure to TCDD and hepta- or octa-PCDDs as well as 2,4-D. The authors noted that both cases of NHL had moderate exposures to 2,4-D and that they had relatively short intervals (3 and 10.5 years) between first exposure to 2,4-D and death.

Lynge (*44*) examined cancer incidence among Danish workers employed in the manufacture of phenoxy herbicides from 1964 through 1982. Cancer cases were identified by linkage with Denmark's National Cancer Register. The cohort was composed of 3390 males and 1069 females who were employed at one of two plants that manufactured herbicides (principally MCPA and, to a lesser extent, other phenoxys including 2,4-D) as well as a variety of other chemicals. Of the 4459 workers, 940 had been assigned to phenoxy herbicide operations. Expected numbers of cancer cases were calculated based on cancer incidence rates in the general Danish population. The highest estimated relative risk (RR) observed was for STS among males: 5 cases were observed versus 1.84 cases expected (estimated RR=2.72; 95% CI 0.88–6.34). Allowing for a 10-year latency period, four cases of STS were observed compared to 1.09 expected (estimated

RR = 3.67; 95% CI 1.0–9.39). Three of the four STS cases had employment periods of 3 months or less, and the cases were not employed in occupations with high potential exposure to 2,4-D. (Only one case had been assigned to chlorophenoxy operations, and his total chemical plant employment was limited to 3 months.) For malignant lymphomas among males, there were 7 cases observed compared to 5.37 expected (estimated RR = 1.30; 95% CI 0.52–2.69). When the author allowed for a 10-year latency period for malignant lymphoma, the estimated RR remained not statistically significant. None of the seven cases of malignant lymphoma occurred in the department producing phenoxy herbicides. The author believes her findings support Hardell's hypothesis of an association between STS and exposure to phenoxy herbicides not contaminated with 2,3,7,8-TCDD, but not Hardell's hypothesis of an association between this exposure and HD or NHL. The study did not report an RR estimate specifically for 2,4-D exposure.

Wiklund et al. (*49*) examined a cohort of 20,245 Swedish pesticide appliers to assess whether HD and NHL are associated with exposure to phenoxy acid herbicides. All appliers had been licensed between 1965 and 1976, and 72% of the appliers were estimated to have been exposed to phenoxy acid herbicides. In Sweden, MCPA was used much more frequently during this period than either 2,4-D or 2,4,5-T. The cohort was followed through the Swedish Cancer Register through 1982. The mean follow-up period was 12.2 years. A total of 11 cases with HD and 21 cases with NHL were observed compared to 9.1 and 20.8 expected. The estimated RRs were 1.20 (95% CI 0.60–2.16) for HD and 1.01 for NHL (95% CI 0.63–1.54). The estimated RRs increased somewhat with increases in the number of years since licensure. Among appliers with 10 or more years since licensure, the estimated RR was 1.16 for NHL (95% CI 0.60–2.02) and 1.45 (95% CI 0.40–3.72) for HD. The authors reported that their RR estimates for NHL did not seem to be consistent with the results reported by Hoar et al. (*40,41*). The major limitations of this study include a relatively short follow-up period, lack of exposure data on individuals, and the absence of RR estimates specifically for 2,4-D exposure.

Considered together, the three cohort studies provide weak to little evidence for an association between exposure to phenoxy herbicides (including 2,4-D) and human cancer. Each cancer type will be discussed separately.

The most provocative result is Lynge's finding of an estimated RR of 3.67 for STS after allowing for a 10-year latency (*44*). However, MCPA and a variety of other chemicals are potential confounding exposures, and no RR estimate is reported specifically for 2,4-D. Moreover, it is noteworthy that three of the STS cases had less than 3 months of work experience at the plant, and several of the STS cases did not hold jobs with a high potential exposure to phenoxy herbicides. Bond et al. (*47*) found no cases of STS in their cohort, although less than one was expected.

The findings for HD in Wiklund et al.(*49*) suggest a weak positive association with use of phenoxy herbicides, but the associations are not statistically significant. Bond et al. (*47*) observed one death from HD in their cohort when less than one was expected. None of the studies reported an RR estimate specifically for 2,4-D exposure, and other chemicals, including other phenoxy herbicides, may have acted as confounders.

The findings for NHL, the end point of greatest interest in the case–control studies, are also unimpressive. Lynge (*44*) observed seven cases of malignant lymphoma, but none occurred among workers in the phenoxy herbicide department. The RR estimates were only slightly in excess, and they did not increase when an adjustment was made for latency. Wiklund et al. (*49*) reported no evidence of an association between phenoxy herbicide use and NHL. Bond et al. (*47*) reported two deaths from NHL under circumstances that seem unlikely to implicate 2,4-D exposure. None of the studies reported an RR estimate for NHL specifically for 2,4-D exposure.

In summary, the cohort studies provide little evidence to suggest that 2,4-D exposure increases the risk for more common types of cancer in humans. Evaluating their findings with regard to the less common cancers is more problematic because of their limited statistical power. These studies will provide more persuasive evidence in the future as the cohorts mature and the length of follow-up for mortality or cancer incidence increases. The workshop participants also noted that the NCI has several large cohort studies of applicator groups underway (i.e., studies of ChemLawn workers and Kansas Noxious Weed Department employees) and that these studies should be expected to contribute substantial information about the human carcinogenicity of 2,4-D.

Weight-of-Evidence Evaluation

There is no single correct way to integrate diverse kinds of data, especially when significant scientific uncertainties exist. In weighing the evidence on the human carcinogenicity of 2,4-D, panel members had some differences in opinion, which we report, but these differences were not very great.

Workshop participants thought that it would be desirable to obtain more detailed information on the levels, duration, and frequency of 2,4-D exposure in the agricultural sector. If use of 2,4-D does cause any health problems, the weight of the available exposure information suggests that the problems will be most evident among those who produce, mix, or apply phenoxy herbicides in occupational settings.

The toxicological data reveal that 2,4-D is a chemical of moderate acute toxicity. Panel members found no indication that the human body's metabolism of 2,4-D produces any particularly toxic metabolites.

2,4-D is unlikely to be a genotoxic carcinogen because it has been shown not to be mutagenic in most *in vitro* and *in vivo* systems. Moreover, the long-term animal bioassays of 2,4-D in mice and rats have not produced impressive evidence of carcinogenicity, although the male rats in the highest dose group experienced a statistically significant increase in the risk of brain tumors relative to controls. Because the results for female rats were negative and because brain tumors tend to have a variable rate of spontaneous incidence in control rats, workshop participants were not convinced that a cause–effect relationship between 2,4-D exposure and brain tumors in rats had been demonstrated. If this end point is demonstrated in the repeat studies, however, the animal carcinogenicity evidence will be much stronger. The weight of the available animal evidence suggests that if 2,4-D is an animal carcinogen, it is probably not a highly potent one.

The recent long-term animal studies of 2,4-D have been criticized for failing to establish and use the maximum tolerated dose (MTD). Workshop participants believed that this criticism

had more merit in the case of the mouse than in the case of the rat. In any event, new animal experiments should be conducted in a manner that resolves the MTD question and provides a rigorous test of the brain tumor hypothesis.

Interestingly, the chronic animal experiments have provided no indication that 2,4-D causes the particular types of cancers that are being explored by epidemiologists. At the same time, there is no epidemiological evidence of an association between use of 2,4-D and brain cancer. Although it might seem that the toxicology and epidemiology of 2,4-D are moving in different directions, there is evidence that human carcinogens do not always have the same target organ that is demonstrated in animal studies (e.g., auramine exposure in humans affects the bladder, but the liver and intestines are the targets in the mouse and rat.) This difference in target organs is not usual, however. Target organs may also vary among different nonhuman species.

The toxicology of 2,4-D alone provides little reason to expect that 2,4-D is carcinogenic in humans. Workshop participants noted that the epidemiology of 2,4-D has arisen out of interest in 2,4,5-T and has been dominated by study of the three cancer types that Hardell (*32,33*) studied originally.

In their weight-of-evidence evaluation, workshop participants gave substantial weight to four case–control studies. The study with the most provocative results found that farmers in Kansas who mixed and applied herbicides themselves had an elevated risk of non-Hodgkin's lymphoma, which increased with days per year of exposure. In a second study in Nebraska (*43*), the same primary investigator and her team collected specific information on the frequency and duration of 2,4-D use and again found an association, albeit weaker, with non-Hodgkin's lymphoma. In both of these studies, farmers who did not adopt standard industrial hygiene practices had an elevated risk of contracting non-Hodgkin's lymphoma. A third study in western Washington did not find an association between phenoxy herbicides and non-Hodgkin's lymphoma, except among applicators who had been exposed for a minimum of 15 years (based on their job title or activities) and for whom at least 15 additional years of a possible latency period had elapsed. No association between 2,4-D use and non-Hodgkin's lymphoma was found in those cohort members who reported using 2,4-D. No data were presented in the Washington study report on days per year of exposure, the variable of most importance in the Kansas and Nebraska studies. A fourth study in New Zealand did not find an association between use of phenoxy herbicides and non-Hodgkin's lymphoma, although the study did not report specific information about 2,4-D. Since the results of the four studies were not identical, judgment was required in weighing the evidence for and against an association. Given factors such as sampling error and the imperfections in such studies described earlier, it is not surprising that the study results differ. The predominant opinion of workshop participants was that the case–control studies, taken together, suggest an association between the use of 2,4-D and non-Hodgkin's lymphoma. Workshop participants did not find evidence from the case–control studies that any other types of cancer are associated with the use of 2,4-D.

Workshop participants also reviewed several cohort studies that found no strong evidence of an association between exposure to 2,4-D and non-Hodgkin's lymphoma. Although these studies were taken into account in the weight of the evidence, they had insufficient follow-up periods and, with the exception of Wiklund et al. (*49*), insufficient sample sizes to negate the association suggested in the case–control studies. As more data on these cohorts is collected in the years ahead, they too may provide more definitive information.

In assessing all of the evidence on 2,4-D, workshop participants were not convinced that a cause–effect relationship between exposure to 2,4-D and human cancer exists. The NCI case–control studies from Kansas and Nebraska suggest an association between frequent occupational use of 2,4-D and non-Hodgkin's lymphoma, but must be interpreted cautiously because *a*) the association with 2,4-D specifically has not been replicated by other investigators; *b*) it is difficult for such studies to isolate which specific herbicide (or other factor) is responsible for the association; *c*) the association may be explained by other chemicals that farmers mix with 2,4-D or with impurities in the 2,4-D that was sold commercially in the post-World War II period; and *d*) use of 2,4-D may be serving as a surrogate in these studies for some other unknown confounding factor. While a cause–effect relationship is far from being established, the evidence for an association between use of 2,4-D and non-Hodgkin's lymphoma is suggestive and requires further investigation. Additional epidemiological studies are underway in the United States, Canada, New Zealand, and Sweden. Panel members stressed the need for future studies to develop more reliable and precise estimates of 2,4-D exposure and to distinguish more clearly between 2,4-D and other agents, including other herbicides, in their data collection, analysis, and reporting of results.

Workshop participants were asked to assess how likely it is, based on the available evidence, that exposure to 2,4-D causes cancer in humans. They were asked to respond with one of five answers: "known carcinogen," "probable carcinogen," "possible carcinogen," "unlikely carcinogen," and "noncarcinogen." These terms were used in their ordinary sense by workshop participants and do not refer to the specific carcinogen classification categories used by the U.S. EPA, IARC, or by any other organization. None of the workshop participants thought the weight of the evidence indicated that 2,4-D was a "known" or "probable" cause of human cancer. Most workshop participants (11 out of 13) responded that the available evidence suggests that it is "possible" that 2,4-D can cause cancer in humans. Not all of these 11 participants thought the possibility was equally likely; one participant thought the possibility was relatively strong, leaning toward "probable" and five thought the possibility was relatively remote, leaning toward "unlikely." A minority of two participants thought, based on the weight of the evidence, that it was unlikely that 2,4-D can cause cancer in humans. Several panel members expressed the opinion that the available evidence was barely adequate to support any conclusion at this time.

In light of the possible cause–effect relationship between the use of 2,4-D and non-Hodgkin's lymphoma, the magnitude of the potential public health problem should be assessed. The age-adjusted incidence of non-Hodgkin's lymphoma is 12 cases per 100,000 person-years, and the highest human exposure levels occur in selected occupational settings. The available evidence also suggests that the risk of contracting non-Hodgkin's lymphoma, assuming a cause–effect relationship with 2,4-D, can be reduced if workers wear protective equipment during mixing and ap-

plying herbicides and change clothing immediately after exposure to herbicides. The panelists were encouraged to learn that manufacturers are currently testing alternative delivery systems for 2,4-D which, if successful, would greatly reduce the potential for applicator exposure.

This report summarizes the work of a panel of 13 scientists assembled by John D. Graham, Director of the Center for Risk Analysis at the Harvard School of Public Health. The panel convened at Belmont (a conference facility in Elkridge, Maryland, owned and operated by the American Chemical Society) on October 17–19, 1989. The project was sponsored by the National Association of Wheat Growers Foundation through a grant from the Industry Task Force II on 2,4-D Research Data. This report was prepared by the project staff at the Center for Risk Analysis and represents the collective opinion of the panel based on the weight of the scientific evidence. The opinions expressed in the report are those of the workshop participants and should not be attributed to either the organizations that employ them or to the Harvard School of Public Health. Research, editorial, and technical assistance were provided by Scott Wolff of Environmental Health Sciences, Inc., in San Francisco, and Emily Schifrin of The Harvard Center for Risk Analysis.

Appendix

Authors and Affiliations

MICHEL A. IBRAHIM
School of Public Health
University of North Carolina at Chapel Hill
Chapel Hill, NC

GREGORY G. BOND
Epidemiology Department
Dow Chemical Company
Midland, MI

THOMAS A. BURKE
The Johns Hopkins School of Hygiene and Public Health
Baltimore, MD

PHILIP COLE
Department of Epidemiology
School of Public Health
University of Alabama at Birmingham
Birmingham, AL

FRANK N. DOST
Department of Agricultural Chemistry
Oregon State University
Corvallis, OR

PHILIP E. ENTERLINE
Department of Biostatistics
University of Pittsburgh School of Public Health
Pittsburgh, PA

MICHAEL GOUGH
Office of Technology Assessment
Washington, DC

RAYMOND S. GREENBERG
Department of Epidemiology and Biostatistics
Emory University School of Medicine
Atlanta, GA

WILLIAM E. HALPERIN
National Institute of Occupational Safety and Health
Cincinnati, OH

EUGENE MCCONNELL
Raleigh, NC

IAN C. MUNRO
Canadian Centre for Toxicology
Guelph, Ontario, Canada

JAMES A. SWENBERG
University of North Carolina at Chapel Hill
Chapel Hill, NC

SHELIA HOAR ZAHM
National Cancer Institute
Rockville, MD

JOHN D. GRAHAM
Center for Risk Analysis
Harvard School of Public Health
Boston, MA

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